

Amendments to the Specification

Please replace the paragraph beginning at page 2, line 15, with the following rewritten paragraph:

--The invention thus encompasses transgenic plants that express at least one dermaseptin or temporin peptide, and methods of making such plants. Parts of such plants, including seeds, fruit, stems, leaves and roots, may be utilized in conventional ways as food sources, or as a source of the dermaseptin or temporin peptides. Because all plant types are susceptible to one or more plant pathogens, the present invention may be usefully applied to produce broad-spectrum resistance in any plant type. Thus, the invention may be applied to both monocotyledonous, dicotyledonous and gymnosperm plants, including, but not limited to maize, wheat, rice, barley, soybean, ~~cotton~~, beans in general, rape/canola, alfalfa, flax, sunflower, safflower, brassica, cotton, flax, peanut, clover; vegetables such as lettuce, tomato, cucurbits, cassava, potato, carrot, radish, pea, lentils, cabbage, cauliflower, broccoli, Brussels sprouts, peppers; tree fruits such as citrus, apples, pears, peaches, apricots, walnuts; and flowers such as orchids ~~orehides~~, carnations and roses.--

Please replace the paragraph beginning at page 3, line 16, with the following rewritten paragraph:

--Other synthetic forms of dermaseptins and temporins that may be employed include forms having N-terminal peptide extensions. Such peptide extensions may comprise portions of the precursor forms of dermaseptins or temporins that are usually removed during protein processing, or may be synthetic sequences. These N-terminal peptide extensions may serve to provide enhanced resistance to proteolytic cleavage, and may also enhance the antimicrobial activity of the peptides. Typically, these N-terminal extensions are of between 2 and 25 amino acids in length, although longer extensions may also be employed. Examples of N-terminal extension sequences that are utilized in certain embodiments include the peptide sequences MAMWK (amino acids 1-5 of SEQ ID NO: 28) and MASRH (amino acids 1-5 of SEQ ID NO: 34). The AMWK sequence (amino acids 1-5 of SEQ ID NO: 28) is a naturally-occurring peptide extension; it is part of the full-length dermaseptin-b peptide sequence that is normally cleaved during processing. The ASRH (amino

acids 1-5 of SEQ ID NO: 34) is a synthetic extension sequence. In each case, the N-terminal methionine is added to the extension peptide to ensure proper expression of the peptide.--

Please replace the paragraph beginning at page 4, line 21, with the following rewritten paragraph:

--Although such pro-region peptides may be directly joined to the N-terminus of the dermaseptin or temporin peptide, it may be beneficial to join the two peptides using a spacer peptide. The use of spacer peptides to join two peptide domains is well known in the art; such spacer peptides are typically of between 2 and 25 amino acids in length, and provide a flexible hinge connecting the first peptide sequence to the second peptide. Spacer sequences that have been used to provide flexible hinges connecting two peptide sequences include the glycine(4) serine spacer (GGGGS x3; SEQ ID NO: 42) described by Chaudhary et al., *Nature* **339**: 394-397, 1989. Alternatively, an N-terminal peptide extension as described above may serve to provide the spacer peptide function. Fusion peptides that comprise a pro-region peptide, a spacer peptide and a dermaseptin or temporin peptide may be represented as P-S-D or P-S-T, wherein S represents the spacer peptide.--

Please add the following new paragraph after the paragraph ending on line 4 of page 6:

--SEQ ID NO: 42 shows the protein sequence of a glycine serine spacer (GGGGS x3).--

Please replace the paragraph beginning at page 9, line 14, with the following rewritten paragraph:

--The dermaseptin peptide is determined to have biological activity if, under the conditions of this assay, it is capable of inhibiting bacterial growth by at least 10% at a concentration of 7 μ g per ml (i.e., at this concentration, the number of bacterial colonies is no more than 90% that of the control plate).--

Please replace the paragraph beginning at page 9, line 29, with the following rewritten paragraph:

--The dermaseptin peptide is determined to have biological activity if, under the conditions of this assay, it is capable of inhibiting fungal growth at a concentration of 5 μ g per

ml (i.e., there is a discernible zone of inhibition around a well containing this concentration of peptide).--

Please replace the paragraph beginning at page 13, line 3, with the following rewritten paragraph:

-- The NCBI Basic Local Alignment Search Tool (BLAST) (Altschul et al., *J. Mol. Biol.* **215**:403-410, 1990) is available from several sources, including the National Center for Biotechnology Information (NCBI, Bethesda, MD) and on the Internet, for use in connection with the sequence analysis programs blastp, blastn, blastx, tblastn and tblastx. ~~It can be accessed at <http://www.ncbi.nlm.nih.gov/BLAST/>. A description of how to determine sequence identity using this program is available at http://www.ncbi.nlm.nih.gov/BLAST/blast_help.html.~~--

Please replace the paragraph beginning at page 18, line 1, with the following rewritten paragraph:

--While the anionic pro-region peptide may be directly joined to the N-terminus of the cationic peptide, an alternative embodiment involves linking the pro-region peptide to the dermaseptin or temporin peptide using a spacer peptide sequence. The use of spacer peptides to join two peptide domains is well known in the art; such spacer peptides are typically of between 2 and 25 amino acids in length, and provide a flexible hinge connecting the first peptide sequence to the second peptide. Spacer sequences that have been used to provide flexible hinges connecting two peptide sequences include the glycine(4)-serine spacer (GGGS x3; SEQ ID NO: 42) described by Chaudhary et al., *Nature* **339**: 394-397, 1989. Alternatively, an N-terminal peptide extension as described below may serve to provide the spacer peptide function. Spacer sequence peptides may also include a cleavage site, such as a peptide sequence recognized and cleaved by a protease, such as Factor Xa. Such sites facilitate removal of the pro-region from the dermaseptin or temporin peptide following purification from plant tissues. The use of anionic pro-region peptides and spacer peptides to express certain cationic peptides in microbial systems is known in the art and described in US Patent 5,593,866 to Hancock.—

Replace the paragraph on page 18, line 16 - page 19, line 3 with the following rewritten paragraph:

In certain embodiments, an N-terminal extension peptide sequence may be added to the dermaseptin or temporin peptide. Such peptide extensions may comprise portions of the precursor forms of dermaseptins or temporins that are usually removed during protein processing, or may be synthetic sequences. These N-terminal peptide extensions may serve to provide enhanced resistance to proteolytic cleavage, enhance transcription levels, or enhance the antimicrobial activity of the peptides. Typically, these N-terminal extensions are of between 2 and 25 amino acids in length, although longer extensions may also be employed. Examples of N-terminal extension sequences that are utilized in certain embodiments include the peptide sequences AMWK (SEQ ID: 39), ASRH (amino acids 2-5 of SEQ ID NO: 34), and ALWK (SEQ ID NO: 41). The AMWK (SEQ ID: 39) sequence is a naturally-occurring peptide extension; it is part of the full-length dermaseptin-b peptide sequence that is normally cleaved during processing. The addition of this sequence to the N-terminus of dermaseptin b (to produce dermaseptin B) has been reported to enhance the in vitro antimicrobial activity of the peptide (Strahilevitz, *Biochemistry*, **33**:10951-10960, 1995). The ASRH (SEG ID: 40), and ALWK (SEQ ID: 41) peptides are synthetic extension sequence. In each case, an N-terminal methionine is added to ensure proper expression of the peptide. One of skill in the art will appreciate that the effect of adding any particular N-terminal extension peptide on the biological activity of the peptide being produced (dermaseptin or temporin) may readily be assessed using the biological activity assay described above.

Please replace the paragraph beginning at page 22, line 26, with the following rewritten paragraph:

--Diseases caused by many pathogens affect a wide variety of plant species. These plants can be monocots, dicots or gymnosperms. Thus, for example, dermaseptins and/or temporin peptides may be introduced into plant species including, but not limited to, maize, wheat, rice, barley, soybean, ~~cotton~~, beans in general, rape/canola, alfalfa, flax, sunflower, safflower, brassica, cotton, tobacco, flax, peanut, clover, cowpea, grapes; vegetables such as lettuce, tomato, cucurbits, cassava, potato, carrot, radish, pea, lentils, cabbage, cauliflower, broccoli, Brussels sprouts, peppers; tree fruits such as citrus, apples, pears, peaches, apricots, walnuts; fur trees such as Douglas fir and loblolly pine, and flowers such as carnations and roses.--

Please replace the paragraph beginning at page 26, line 24, with the following rewritten paragraph:

--A nucleic acid molecule was designed to encode the mature 27 amino acid form of dermaseptin b (SEQ ID: 3) with a 5 amino acid N-terminal extension sequence, MAMWK (amino acids 1-5 of SEQ ID NO: 28). This nucleic acid construct was designated MSRA₂ and was synthesized using four overlapping oligonucleotides in a single PCR reaction. The oligonucleotides used are shown in Table 2. The first two oligonucleotides (oligo #1 and oligo #2) contained the nucleic acid sequences encoding the N-terminal and the C-terminal portions of the peptide, respectively. These oligonucleotides were used in the PCR reaction at a 20 nM concentration. The second two oligonucleotides contained sequences recognized by various restriction enzymes. Specifically, oligo #3 contained restriction sites for *Xba*I, *Kpn*I and *Nco*I, and oligo #4 contained restriction site for *Sst*I, *Pst*I and *Hind*III. These oligonucleotides were used at a concentration of 200 nM in the PCR reaction. Following amplification of the product, it was cloned using the built-in restriction sites into a conventional cloning vector. The nucleic acid sequence of the coding region of MSRA₂ is shown in SEQ ID: 27, and the encoded peptide is shown in SEQ ID: 28. Oligo #s 1-4 are shown in SEQ IDs: 29-32.

Please replace the paragraph beginning at page 27, line 28, with the following rewritten paragraph:

--A nucleic acid molecule was designed to encode the mature 13 amino acid form of temporin A (SEQ ID:33) with a 6 amino acid N-terminal extension sequence, MASRHM (amino acids 1-6 of SEQ ID NO: 34). This nucleic acid construct was designated MSRA₃ and was synthesized using four overlapping oligonucleotides in a single PCR reaction. The oligonucleotides used are shown in Table 3. The first two oligonucleotides (oligo #1 and oligo #2) contained the nucleic acid sequences encoding the prototype peptide. However, unlike the oligonucleotides used to encode the prototype dermaseptin peptide, these oligonucleotides were fully complementary, thus eliminating the need for an initial elongation cycle prior to the binding of oligos #3 and #4. Oligos #1 and #2 were used in the PCR reaction at a 20 nM concentration. The second two oligonucleotides contained sequences recognized by various restriction enzymes. Specifically, oligo #3 contained restriction sites for *Xba*I, *Kpn*I and *Nde*I, and oligo #4

contained restriction site for *Sst*I, and *Pst*I. These oligonucleotides were used at a concentration of 200 nM in the PCR reaction. --

Please replace the paragraph beginning at page 29, line 1, with the following rewritten paragraph:

--2. Vectors ~~C~~ontaining Containing Various Promoter Sequences--

Please replace the paragraph beginning at page 29, line 14, with the following rewritten paragraph:

--Another vector was designed such that the MSRA₂ construct was under the control of a rebuilt "super promoter". This promoter contained the mas (mannopine synthase) promoter/activator region (Langridge et al., *Bio/Technology* 10:305-308, 1989) preceded by a trimer of ocs (octopine synthase) upstream activating sequence (in inverted orientation). A more detailed description of this super promoter is provided in Ni et al., *The Plant J.*, 7: 661-676, 1995. This particular construct also contained a coding region for a 6 x His tag, positioned upstream of and operably linked to the MSRA₂ coding region. The 6 x His tag amino acid sequence is thus expressed as an N-terminal addition to the encoded dermaseptin/ MAMWK (SEQ ID NO:28) peptide. The vector encoding this peptide was designated pRSHMSRA₂.--

Please replace pages 1-11 of the previous sequence listing with pages 1-11 of the enclosed sequence listing.

Enclosure: 11 pages of sequence listing.